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Chemical Constituents and *in Vitro* Biological Screening of Hydroacetonic Extracts of 3 African Toothpicks

Amani Brice Kadja[1*], Yves-Alain Békro[1], Jean-Luc Pirat[2], Janat Akhanovna Mamyrbékova-Békro[1], Jean-Noël Volle[2], Marc Lecouvey[3] and Marie-Hélène Boyer-Lavergne[4]

[1]Laboratoire de Chimie Bio-Organique et de Substances Naturelles/ UFR-SFA/ Université Nangui Abrogoua, 02 BP 801 Abidjan 02.

[2]UMR 5253, ICG Montpellier, Equipe AM2N, ENSCM, 8, rue de l'Ecole Normale, 34296 Montpellier cedex 5, France.

[3]Equipe Chimie Bio-organique et structurale/Université Paris 13Nord.

[4]Laboratoire de microbiologie, Université de Montpellier.

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Abstract: The chemical and biological survey has been achieved on the stems of *Erythrophleum africanum* (Fabaceae), *Terminalia albida* (Combretaceae) and *Zanthoxylum zanthoxyloides* (Rutaceae), from 3 African plants used as toothpicks. The mineral quantification using the scanning electron microscope, highlight the presence of Al, Cl, Fe, K, Mg, Mn, P, S, Si with a relative abundance in K and Ca, when the organic characterization by UPLC revealed the existence of phytophenols notably epigallocatechin gallate (EGCG), epicatechin gallate (ECG), chlorogenic acid, gallic acid, burkinabines, ellagitannins). Compared to vitamin C and butylhydroxytoluene (BHT), hydroacetonic extract from *E. africanum* stem exhibited a significant anti-radical power (92.19 %) with regard to DPPH. The biological screening of the aforesaid extract showed an antibacterial activity against *Staphylococcus aureus* and *Micrococcus luteus*. The hydroacetonic extracts from *E. africanum*, *T. albida* and *Z. zanthoxyloides* stems signed an activity with regard to 6 cancerous strains MDA-MB 231, Caco-2s, B16F10, MDA-MB 435, SNB75 and C6, with a good antiproliferative profile opposite Caco-2, SNB75 and of C6. Among the extracts of the drugs tested, the

one of *Z. zanthoxyloides* stem made notice a more important effect on all cancerous strains.

Keywords: African toothpick, phytochemical analysis, biological screening, *in vitro*.

INTRODUCTION

The toothpicks are part of medicinal plants known for their antioxidant power and effectiveness against fungal, viral, bacterial and cancer[1,2] due to their mineral composition and phenolic components. Ethnobotanical surveys conducted in Sikasso (Mali) were used to identify 3 plants namely *Erythrophleum africanum* (Fabaceae), *Terminalia albida* (Combretaceae) and *zanthoxyloides Zanthoxylum* (Rutaceae), which stems are mainly used not only in the mouth clean but also in maintenance the prevention and/or treatment of certain diseases such as (bacterial infections, cancer, ulcers, malaria, stomach ache, sexual impotence). These three plant species have already been subject of several studies[2-8]. However, investigations on the phytochemistry and the biology of stems of the aforesaid plants are poorly documented. This study is a contribution to the valorization of the use of the African plant toothbrushes as oral toilet utensils.

MATERIALS AND METHODS

Material plant: The plant material of survey concerned the stems of *Erythrophleum africanum*[3,4], *Terminalia albida* and of *Zanthoxylum zanthoxyloides*, harvested in Longorola in Sikasso (Mali) in 2010 then authenticated at the Centre National de Floristique (CNF) of Félix Houphouët-Boigny University (Abidjan-Cocody).

Biological material: The biological material used contains 4 bacterial strains including *Escherichia coli*, *Pseudomonas aeruginosa* (Gram negative) and *Staphylococcus aureus*, *Micrococcus luteus* (Gram positive) and 6 Referenced cancer stem MDA-MB 231 (breast human cancer cell line), Caco2 (line human cancer of the colon), B16F10 and MDA-MB 435 (malignant melanoma lines), SNB75 (cancerous line of the brain) and C6 (cancerous line derived from rat glioblastoma).

Mineral quantification: The mineral quantification study of the plants stems was carried out using the Scanning Electron Microscope (SEM) with Variable Pressure of D.C.AR. (SEM FEG Zeiss Supra 40 VP), equipped with an X-ray detector (Oxford Instruments) connected to EDS microanalysis platform (Inca Dry Cool, without liquid nitrogen)[4].

UPLC qualitative analysis: The phenolic composition of aforesaid plant organs has been determined by liquid chromatography Ultra High Performance (UPLC) analysis[4].

Anti-radical test: Plant stems anti-radical activity against DPPH have been evaluated by comparison with vitamin C and butylhydroxytoluene (BHT)[3].

In vitro biological activity

Anti-bacterial test: Antibacterial activity assessment of the hydroacetonic excerpts of the stems has been achieved on the one hand, by diffusion on agar and on the other hand, on microtiter plate in the middle Muller Hinton. The antibacterial activity has been evaluated while measuring the diameter of the inhibition zone. The MIC (Inhibitory Minimal Concentration) has been determined for every antibacterial. The BMC (Bactericidal Minimal Concentration) has been determined by taking 10 µL in each well presenting a growth inhibition , deposited on a Lysogeny Broth agar box incubated all night long at 37°C.

Anti-cancer test: The culture medium used is an "Eagle" medium modified by Dulbecco (DMEM) (Sigma) supplemented with 10% (v/v) fetal calf serum (FCS) source of essential growth factors and 1% (v/v) Penicillin Streptomycin. Anticancer activity *in vitro* evaluation has been on a microtiter plate, which was incubated at 37°C in an atmosphere enriched with CO_2 (5%) for 24h. Measuring mitochondrial activity of the cells after treatment with the selected dose of plant extracts compared with mitochondrial activity of untreated cells, allowed to indirectly evaluate the activity of various extracts. The percent viability is calculated using the equation:

PV (%) = (Abs test / Abs Witness) × 100

PV: cell viability percentage; **Abs test**: the test absorbance treated extracts; **Abs Witness**: absorbance of the untreated test by extracts

STATISTICAL ANALYSIS

All experiments were performed in triplicate and results are expressed as mean ± standard deviation using SAS software[9].

RESULTS AND DISCUSSION

Mineral contents: Figure 1 indicated shows the quantitative mineral profile each plant species. Indeed, mineral elements C, Ca, Cl, Fe, K, Mg, Al, Si, P and S are present with a net respective abundance of Ca and K (12.02% and 21.12% in *E. africanum*, 18.87% and 17.65% in *T. albida*, 21.70% and 13.63% for *Z. zanthoxyloides*).

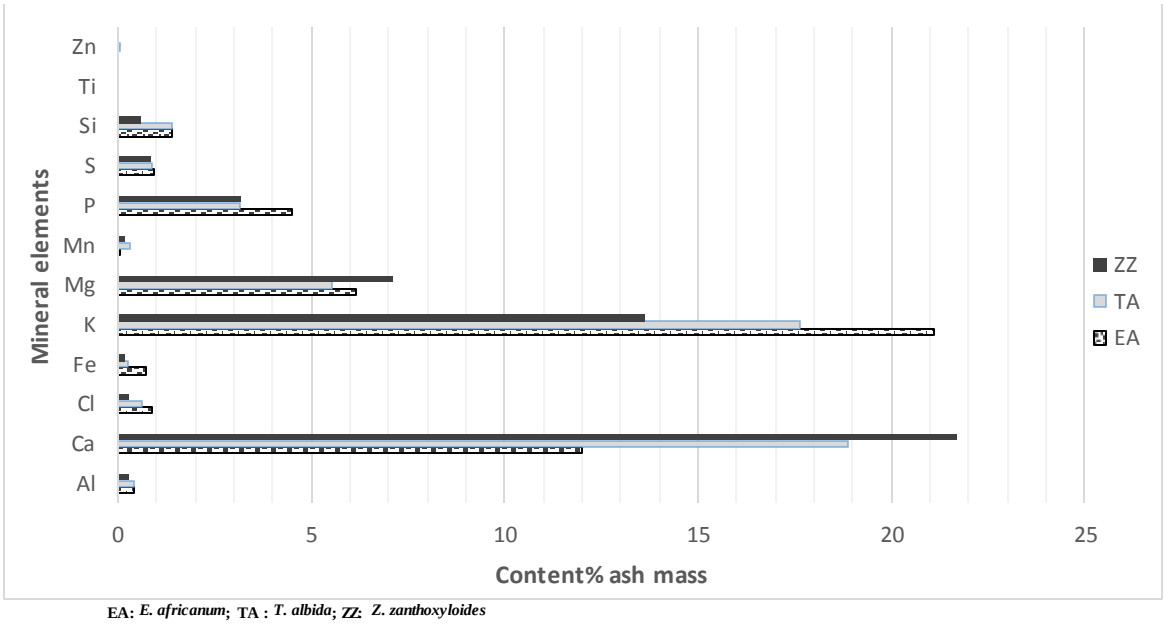


Fig. 1: Quantitative mineral profile of each plant stem

E. africanum stem was revealed the richest in K and P. As for that the one of *Z. zanthoxyloides*, it contains Ca and Mg. Otherwise, the presence of Zn and Ti is noticed but in very weak respectively in *T. albida* (0.06%) and *Z. zanthoxyloides* (0.04%). In general, minerals are essential nutrients that come from the external environment[10] and remain after calcination of organic tissues. These are indispensable biocatalysts to the harmonious functioning of protein, enzymatic and genetic systems. For example, the main function of Ca is its participation in the edification and the renewal of the skeleton, and teeth

formation[11] and is also involved in blood clotting to muscle and cardiac contraction, cellular exchanges, membrane permeability, the hormone release and transmission of nerve impulses. Thus it is reported that the minerals help protect against oxidative stress[12].

UPLC phenolic composition: The chromatographic profile analysis of hydracetonic extract from *E. africanum* stem of, revealed the presence of phenolic compounds such as gallic acid, epigallocatechin gallate (EGCG) and epicatechin gallate (ECG)[4]. Figures 2 and 3 show the chromatograms of hydroacetonic aqueous-acetone extracts from *T. albida* and *Z. zanthoxyloides* stems respectively.

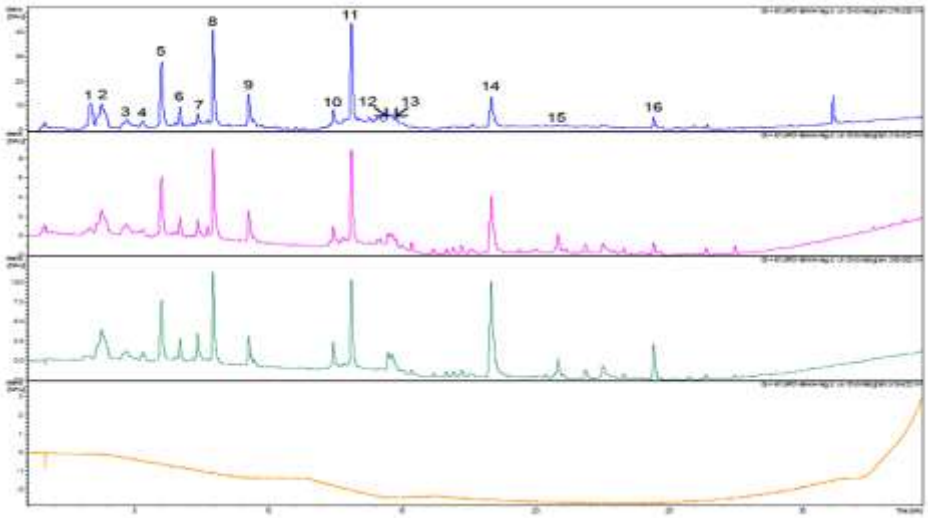


Fig. 2: Chromatographic profiles of hydroacetonic extract from *T. albida* stem

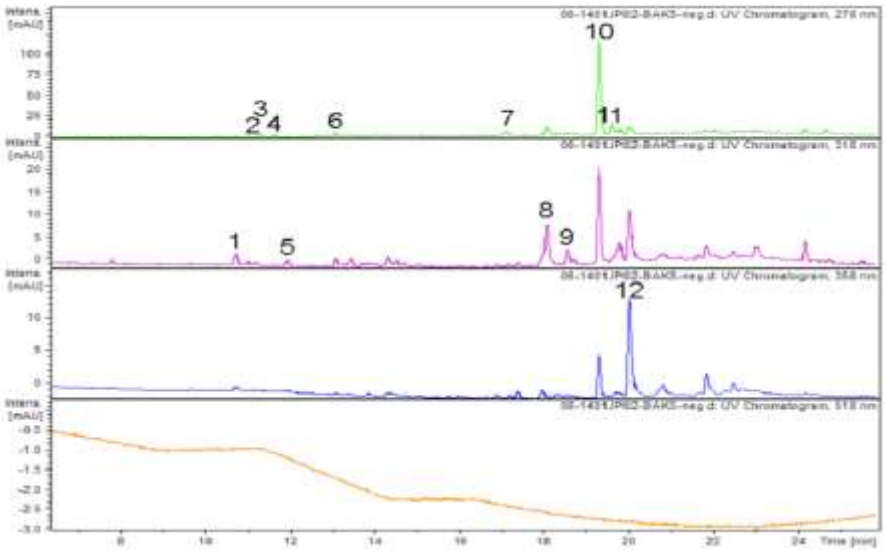


Fig. 3: Chromatographic profile of hydroacetonic extract from *Z. zanthoxyloides* stem

All phytocompounds families that may be contained in the hydroacetonic extracts is recorded in Tables 1 and 2.

Table 1: Possible phenolic compounds detected in *T. albida*

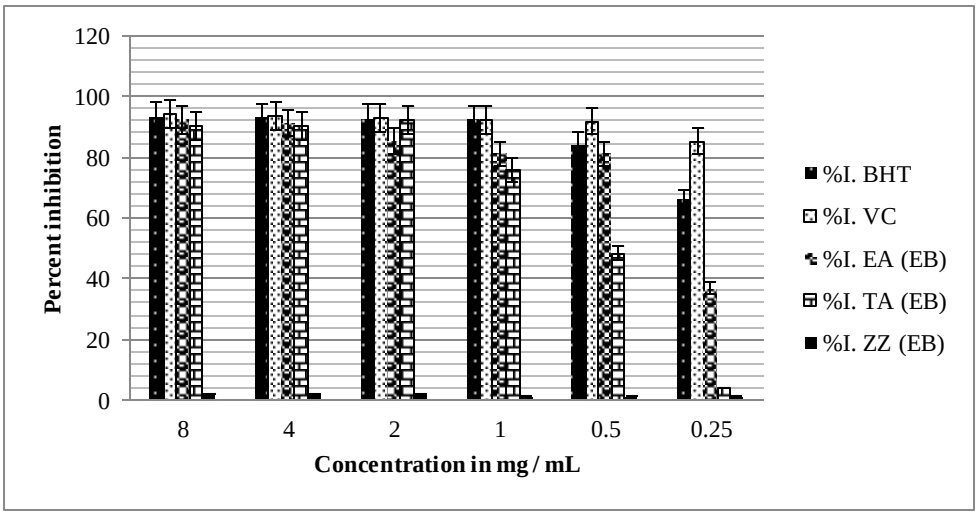
Peak	Tr (min)	λ max (nm)	Molar mass	MS (m/z) ; Ion + / -	MS, MSn	possible compound
1	3,3	280	170	$[M-H]^-$ = 169	127	Gallic acid
2,3	3,8 ; 4,7	360	782,52	$[M+H]^+$ = 783	765, 603, 421	Punicalin
4	5,3	360	934	$[M-H]^-$ = 933	721,600	Castalagin
5	6	360	1084,71	$[M+H]^+$=1085		not determined
6,7	6,7 ; 7,3	360	782,52	$[M+H]^+$ = 783	765, 603, 421	Punicalin
8,9,10,11	7,9; 9,2; 12,4; 13,1	360	1084,71	$[M+H]^+$=1085	905, 567	Punicalagin
12	14,5	360	954,66			not determined
13	14,6	360	601			not determined
14	18,3	360	302	$[M-H]^-$ = 301	257, 229	ellagic acid
15	20,8	360	356	$[M-H]^-$ = 355		not determined
16	24,4	360	344	$[M+H]^+$ = 345	191, 152	galloyquinic acid

Table 2: Possible phenolic compounds detected in *Z. zanthoxyloides*

Peak	Tr (min)	λ max (nm)	Molar mass	MS (m/z) Ion + / -	MS, MSn	possible compound
1	10,7	320	354	$[M-H]^-$= 353	180	chlorogenic acid
2	11	280	342	$[M-H]^-$ = 341	323, 173	phenol acid
3	11,3	280	444	$[M-H]^-$= 443	279, 356	phenol acid
4	11,6	280	372	$[M-H]^-$ = 371	191	phenol acid
5	12	320	354	$[M-H]^-$ = 353	180	chlorogenic acid
6	13,1	280	341	$[M]^-$ = 537	491, 441, 307	phenol acid (derivative of vanillic acid)
7	17,1	280	492	$[M-H]^-$ = 491	323, 168	Burkinabine A
8	18,1	320	492	$[M-H]^-$ = 491	323, 168	Burkinabine B
9	18,6	320	343	$[M]^-$ = 495	450, 403, 342	hydroxycinnamic acid
10	19,3	280	610	$[M-H]^-$ = 609	301, 308, 286	Flavanone (neohesperidine)
11	19,6	280	492	$[M-H]^-$ = 491	323, 168	Burkinabine C
12	20	360	302	$[M-H]^-$ = 607	301, 308	Flavone

Anti-radical activity: BHT and VC are two referenced antioxidants that showed a very significant DPPH free radical scavenging activity in the interval of concentrations [0.00025-0.008 g/mL]. The Hydroacetonic crude extracts (EB) of plants stem, exhibit a good anti-radical activity 0.008 g/mL, except that of *Z. zanthoxyloides* which the inhibition percentage is distinctly lower to 50% (Figure 4). Similarly, we note to this concentration that EB of *E. africanum* and *T. albida* exhibit an inhibition percentage 92.19% and 90.08% respectively, appreciably equal to the two referenced antioxidants. This propriety

is also observed until 0.001 g/mL, which seems to bind to the composition of these extracts. Indeed, stems *E. africanum* and *T. albida* contain gallic acid and hydrolyzable tannins among other castalagin. It is documented that the derivatives of gallic acid are bioactive and potentially antioxidants[13]. To 0.0005 g/mL, only EB of *E. africanum* keeps its antioxidant character, what reveals its high content of phytophenols (934.2 µgEAg/g)[4, 14].



BHT : Butylhydroxytoluene ; VC : Vitamin C ; EA: *E. africanum* ; TA : *T. albida* ; ZZ : *Z. zan-thoxyloides*

Fig. 4: DPPH radical antioxidant scavenging activity of hydroacetonic extracts from plants stems

Anti-bacterial activity: The antibacterial test showed a bacterial inhibition EB against the 4 tested strains (Table 3).

Table 3: diameters of inhibition zones

	EB		
Bacterial strain	**EA**	**TA**	**ZZ**
E. coli	0	0	0
P. aeruginosa	0	0	0
S. aureus	10	0	13
M. luteus	19	12	0

0 = absence of inhibition halo; diameter of inhibition halo (in mm); EB: Hydroacetonic crude extracts; EA: *E. africanum*; TA: *T. albida*; ZZ: *Z. zanthoxyloides*

E. coli and *P. aeruginosa* are resistant against EB EA, TA and ZZ as the absence of inhibition diameter attests it (Table 3). This resistance would probably explain itself by the structural nature of the cell wall of gram positive and gram negative bacteria[15]. The table 4 reveals the type of activity of each vis-a-vis extract of the bacterial strains and that, to the look of determined antibacterial parameters MIC and CMB. According Marmonier[16], is a substance said bactericidal so BMC/MIC $\leq$ 4 and bacteriostatic so BMC/MIC > 4.

Table 4: Antibacterial parameters of extracts tested

bacterial strain	EB	MIC, µg/mL	BMC, µg/mL	BMC/MIC	Activity
S. aureus	EA	156	312	2	bactericidal
	TA	ND	625	ND	ND
	ZZ	ND	ND	ND	ND
M. luteus	EA	78	78	1	bactericidal
	TA	39	625	16	bacteriostatic
	ZZ	ND	ND	ND	ND

ND: not determined; EB: Hydroacetonic crude extracts; EA: *E. africanum*; TA: *T. albida*; ZZ: *Z. zanthoxyloides*

We note indeed, that EB of EA signs bactericidal activity against *S. aureus* and *M. luteus*. A hypothesis can be given out to explain the action of EA to the look of its composition in phytophenols notably in tannins. A survey achieved by Weiss[17] demonstrated that an integrated phenolic fraction mouth bath can entail a meaningful reduction of *S. mutans*. From where to pay a particular attention to the phenolic secondary metabolites.

Anti-cancer activity: The percentages of viability of the different cancerous stumps after treatment of 72 h with a range of active concentrations of 2 to 0.125 mg/mL of plants EB, have been determined. They are for each of the strains and nearly too all concentrations of *Z. zanthoxyloides*, less than 50%. A 0.5 mg/mL, the different extracts from stems make see a meaningful toxicity against various cancerous strains. With regard to C6, viability percentages are all lower to 50%; what would reflect a manifest antiproliferative activity of plant samples analyzed. The statistical treatment of viability percentages (Table 5) shows the global effect of EB plants noted on all tested cancerous strains.

Table 5: Global effect of plants EB on the cancerous strains

Sample	N	mean±standard deviation	F	P
EA	90	51,139 ± 25,384	21,72	< 0,0001
TA	90	44,208 ± 25,563		
ZZ	90	20,720 ± 16,573		

EB of *Z. zanthoxyloides* clears itself distinctly with a weak percentage of survival low (20.720 ± 16.573) of the cancerous cells for a threshold $\alpha = 0.0001\%$. So it appears that the most active therefore on the all tested cancerous strains.

CONCLUSION

From the present scientific survey, we meaningfully contributed to the valorization of *Erythrophleum africanum*, *Terminalia albida* and *Zanthoxylum zanthoxyloides*, 3 African plants to pharmacological properties of which the use of the stems is recurrent in oral hygiene. The mineral analysis of stem of the aforesaid analyzed plants, made come out again approximately, two types of minerals manely the major minerals (Ca, Cl, K, Mg, P) that represent a part important of the content of the organism and the oligoelements (Fe, Mn.) necessary to the good organism functioning. K and Ca elements are respectively present in all plants in major content. UPLC chemical characterization of the excerpts of stems of the plants made note the presence of phenolic secondary metabolites among which EGCG, EKG, chlorogenic acid, gallic acid burkinabines, ellagitannins. The plants antioxidant potential has been

valued spectrophotometrically by scavenging DPPH, in comparison with those of vitamin C and BHT. The plants expressed on the whole, a good anti-radical behavior. With regard to the biologic tests, the antibacterial and anti-proliferative profiles of the plants have been shown. These results are a preliminary step in the research for natural treatment that opens an explanatory track of the recurrent use of some African plants like oral toilet utensil.

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***Corresponding Author: Amani Brice Kadja;**

Laboratoire de Chimie Bio-Organique et de Substances Naturelles/ UFR-SFA/ Université Nangui Abrogoua, 02 BP 801 Abidjan 02, France.